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IONONE DISINFECTANT

Abstract:

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(A1) A disinfectant composition containing an ionone and another terpene that is effective against several types of bacteria and a broad range of fungi. In a preferred embodiment, the disinfectant composition comprises about 45% ionone, about 40% another terpene, about 20% surfactant, and about 5% isopropyl alcohol, all expressed by volume. As a foot bath, the composition is diluted with water about 1 to 1,000, and as a spray, it is diluted with water or organic solvent about 1:1 to 1:100. Preferred ionones are beta ionone and pseudo-ionone. Preferred other terpenes included DL-limonene, dipentene, citral, terpineol, and pinene.

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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Ionone Disinfectant

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ABSTRACT OF THE DISCLOSURE

A disinfectant composition containing an ionone and another terpene that is effective against several types of bacteria and a broad range of fungi. In a preferred embodiment, the disinfectant composition comprises about 45% ionone, about 40% another terpene, about 20% surfactant, and about 5% isopropyl alcohol, all expressed by volume. As a foot bath, the composition is diluted with water about 1 to 1,000, and as a spray, it is diluted with water or organic solvent about 1:1 to 1:100. Preferred ionones are beta ionone and pseudo-ionone. Preferred other terpenes included DL-limonene, dipentene, citral, terpineol, and pinene.

BACKGROUND OF THE INVENTION

Field of the Invention.

The present invention relates to a safe, biodegradable disinfectant that is easy to handle and use, while exhibiting good efficacy against a variety of bacteria and fungi. Broadly stated, the ionone disinfectant of this invention is a composition comprising from about 1% to about 70% beta ionone or pseudo-ionone, from about 1% to about 70% DL-limonene, dipentene, citral, terpineol, or pinene, from about 1% to about 30% surfactant, and about 1-10% isopropyl or ethyl alcohol, all by volume. The disinfectant composition is a substantially clear liquid and is diluted with water for use as a foot bath for animals or for spraying.

Description of the Prior Art.

Both the dairy industry and the veterinary profession have long recognized that foot rot is a hard-to-treat condition that can remove cattle from production for extended periods of time. While a variety of methods have been used to prevent foot rot, most have met with only limited success. One formulation generally acknowledged as useful for treating foot rot and other animal hoof problems is an ionized copper solution made by SSI Corporation and sold under the trademark "HOOFPRO+."

However, that product and other similar products have met with only limited success. It remains clear that a need for treating such hoof diseases remains, and that treatment compositions must not only exhibit efficacy, but also must be economical and environmentally-safe. Accordingly, attention has turned to other bactericides and fungicides.

4,963,583 and U. S. Patent No. 5,001,155 both teach the use of beta-ionone derivatives as anti-fungal agents. U. S. Patent No. 4,474,816 teaches a method for inhibiting aflatoxin produced by strains of *Aspergillus parasiticus* fungi by the application of beta-ionone. U. S. Patent No. 4,814,163 discloses an antitartar mouth deodorant comprising a zinc compound, an ionone ketone terpene derivative, and a mint flavor as the essential active ingredients. Yet another antitartar mouth deodorant comprising a zinc compound, an ionone ketone terpene derivative and a flavor as the essential active ingredients is disclosed in U. S. Patent No. 4,814,164.

Thus, while the prior art teaches the use of terpenes and/or terpene derivatives as active ingredients for controlling some bacteria and fungi, there is no teaching in the prior art of such a disinfectant composition useful in the veterinary profession for the control of bacteria- and fungi-related foot diseases.

One class of compounds considered has been terpene-based mixtures previously well known for use as industrial cleaning agents. The use of terpene derivatives as antifungal agents is well known and is taught in prior patent literature. For example, U. S. Patent No.

SUMMARY OF THE INVENTION

The present invention relates to a disinfectant composition containing an ionone and another terpene which is especially efficacious against several types of bacteria and fungi. According to a preferred formulation, the disinfectant composition comprises about 45%, by volume, beta-ionone or pseudo-ionone, plus about 40%, by volume, DL-limonene, plus about 10%, by volume, surfactant, plus about 5%, by volume, isopropyl alcohol. The composition of this invention is safe, biodegradable, easy to handle and use, and creates no significant environmental concerns.

The preferred composition described above is added to water for use as a foot bath for animals, or can be sprayed directly on areas to be disinfected. As is set forth in greater detail below, the disinfectant composition has been found to be effective against a broad range of both bacteria and fungi, there being very few non-toxic compounds that can achieve this result.

A preferred method for preparing the disinfectant composition of this invention is also described below.

The invention accordingly comprises the several steps and the relation of one or more of such steps with respect to each of the others, and the composition possessing the features, properties, and the relation of constituents which are exemplified in the following detailed disclosure, and the scope of the invention will be indicated in the claims.

DETAILED DESCRIPTION

Through laboratory tests it was discovered that both beta-ionone and pseudo-ionone begin to have an effect against several types of bacteria at approximately 20 ppm, and that greater than 100 ppm give nearly 100% kill of both bacteria and fungi. This is significant because it is far less than the toxic level (LD_{50} in mice is greater than 5 g/kg) and far below a level that would normally be expected to cause eradication of the bacteria and fungi. At this point, experiments were conducted to maximize the efficacy and to create a practical and usable format for the ionones. At this point it should be noted that alpha-ionone was found to have almost no efficacy.

Because of the safety of beta-ionone and pseudo-ionone, and because of the necessity of delivering the active ingredients in a water solution, it was next determined that a non-ionic surfactant having an HLB range of 10 to 18 was necessary. The preferred surfactant is polysorbate.

Further laboratory tests confirmed that the addition of another terpene would increase efficacy when used in combination with either the beta-ionone or the pseudo-ionone. Of the other terpenes tested, it was determined that DL-limonene, dipentene, citral, terpineol, or pinene were all effective. However, DL-limonene was chosen because of its "generally regarded as safe" status.

Inasmuch as the final mixture of beta-ionone or pseudo-ionone with DL-limonene and polysorbate forms a cloudy mixture, isopropyl alcohol was added to give a clear appearance. The isopropyl alcohol appears to have no bearing on the activity of the two principal active ingredients, but may contribute slightly to the disinfectant properties of the composition.

Most generally stated, the disinfectant composition of this invention comprises from about 1% to about 70%, by volume, beta-ionone or pseudo-ionone, from about 1% to about 70%, by volume, other terpene (preferably DL-limonene), from about 1% to about 30%, by volume, non-ionic surfactant having an HLB of about 10 to about 18, and about 1-10%, by volume, isopropyl or ethyl alcohol. In a preferred composition, the invention comprises about 45%, by volume, beta-ionone, about 40%, by volume, DL-limonene, about 10%, by volume, polysorbate 80, and about 5% isopropyl alcohol.

For use as a foot bath, the disinfectant composition is added to water at about a 1 to 1,000 dilution. For spraying on areas to be disinfected, the disinfectant composition is diluted with water or organic solvents at a range of 1:1 to 1:100.

A preferred method for preparing the disinfectant composition comprises the steps of adding the ionone to a mixing vessel maintained at about 20-25°C, with mixing, next adding about 10%, by volume, surfactant with additional mixing for about 60 minutes, then adding the isopropyl alcohol at a rate of about 5-15 liters per minute with additional mixing for about 40 minutes, then slowly adding about 40%, by volume, other terpene with additional mixing while maintaining a substantially clear mixture, and finally blending the final mixture for about 1½ hours to obtain the disinfectant composition.

EXAMPLE I

Utilizing the procedure outlined above, a disinfectant composition was prepared comprising the following ingredients:

INGREDIENT	VOLUME PERCENT
beta-ionone	45
surfactant (tween 80)	10
Isopropyl alcohol	5
DL-limonene	40.

This composition, after mixing, was dispensed into bottles in 240 ml aliquots. An aliquot was tested for pH by placing 0.2 ml of the formula in 50 ml of deionized water. The pH was 5.6. Ten (10) ml of material was measured, and the specific gravity was determined as 0.901.

Standard preparations of *Candida krusei* were grown in tryptic soy broth at 25°C. Standard preparations of *Bacillus subtilus* were prepared in fluid thioglycollate at 35°C. Tenfold dilution blanks from 10^{-1} to 10^{-10} were made in the relevant growth media. The undilute tubes were inoculated with a final concentration of the disinfectant composition set forth above. The undilute bacteria and fungi were subjected to the disinfectant composition for 20 minutes at 25°C or for 20 minutes at 4°C. The tubes of undilute bacteria were diluted in the dilution blanks. The tryptic soy broth was incubated at 20-25°C and the fluid thioglycollate at 35°C, both for four days. The tubes were then macroscopically observed. The results are set forth in Table I. following.

TABLE I

TEST	SAMPLE	TEMP OF MATL	ORGANISM	MEDIA	-1	-2	-3	-4	-5	-6	-7	-8	-9	-10
1	B-ionone	20 min @ 25°C	*B. Subtilus*	Thio	-	-	-	-	-	-	-	-	-	-
2	Control	20 min @ 25°C	*B. Subtilus*	Thio	+	+	+	+	-	-	-	-	-	-
3	B-ionone	20 min @ 4°C	*B. Subtilus*	Thio	-	-	-	-	-	-	-	-	-	-
4	Control	20 min @ 4°C	*B. Subtilus*	Thio	+	+	+	+	-	-	-	-	-	-
5	B-ionone	20 min @ 25°C	*C. Krusei*	SCD	+	-	-	-	-	-	-	-	-	-
6	Control	20 min @ 25°C	*C. Krusei*	SCD	+	+	+	+	+	-	-	-	-	-
7	B-ionone	20 min @ 4°C	*C. Krusei*	SCD	-	-	-	-	-	-	-	-	-	-
8	Control	20 min @ 4°C	*C. Krusei*	SCD	+	+	+	+	+	-	-	-	-	-

At a 1:500 dilution, the disinfectant composition of this invention was bactericidal for *B. subtilus* when exposed for 20 minutes at either 4° or 25°C, and was fungicidal for *C. krusei* when exposed for 20 minutes at either 4° or 25°C. In all cases, a 4 log reduction was observed.

EXAMPLE II

The disinfectant composition was further subjected to other bacteria to determine its efficacy on those organisms.

Staphylococcus aureus, Bacillus subtilus, Salmonella typhimurium, Pseudomonas aeruginosa, and *Candida krusei* were propagated in fluid thioglycollate or tryptic soy broth as required by the organism growth characteristics. A 1:500 dilution of the disinfectant composition of Example I was placed with equal amounts by volume of the organisms in individual tubes. A positive control for each organism was diluted 1:2. Accordingly, the working solution was a 1:1000 dilution, or about 360 ppm of beta-ionone. Both the control tubes and the beta-ionone-containing tubes were incubated at 4°C for 20 minutes and then diluted tenfold in appropriate growth media. The dilutions were incubated at either 20-25°C or 35°C relevant to the media and the organism. The tubes were then read for growth 48 hours after incubation with the following results:

TABLE II

ORGANISM	CONTROL	TEST	REDUCTION
Candida krusei	10^{-4}	10^{0}	99.99%/4 logs
Bacillus subtilus	10^{-4}	10^{-2}	99%/2 logs
Staphylococcus aureus	10^{-6}	10^{-4}	99%/2 logs
Pseudomonas aeruginosa	10^{-5}	10^{-4}	90%/1 log
Salmonella typhimurium	10^{-7}	10^{-6}	90%/1 log

The efficacy of the disinfectant composition against these organisms is apparent. Thus. at a twofold dilution greater than the study reported in Example I, the beta-ionone is significant in reducing organism numbers even at a relatively cool 4°C temperature.

EXAMPLE III

Inasmuch as hairy foot wart may be caused by a spirochete, efficacy of the disinfectant composition of this invention for use in a foot bath killing spirochetes would be a great benefit. The following test was conducted to determine the effect of the disinfectant composition on *Leptospira canicola*.

Leptospira interrogans, serovar canicola (Batch No. 89/02 from ATCC) was propagated in 1 X SPL leptospira growth media (301E1210) at 29°C. The organism was passed in growth media and the culture was utilized 10 days later. There was no agitation of the culture during incubation. The 10 day old culture was sampled and diluted in media. The culture was 100% viable as demonstrated by motility as viewed by darkfield microscopy at 400 power. The culture was quantitated in a Petroff Hausser and Helber Counting Chamber. The resulting culture count was 3.2×10^6 organisms per milliliter.

The disinfectant composition of Example I was diluted 1:10 in water and mixed thoroughly. Then, 0.1 ml of the dilution was added to 10 ml of the active leptospira culture. Samples were taken at 1 minute, 3 minutes 45 seconds, 5 minutes, and 8 minutes post exposure. The samples were viewed and rated by darkfield microscopy as to viability with the following results:

TABLE III

Time of Exposure	% Reduction in Viability	
	Test	Control
1 minute	50%	0%
3 minutes, 45 seconds	90%	0%
5 minutes	95%	0%
8 minutes	100%	0%

At 8 minutes, the remaining sample was added to 100 ml of new growth media for any residual live Leptospira. The sample was incubated for 10 days at 29°C. No growth occurred, indicating complete inactivation.

EXAMPLE IV

An outside laboratory was provided with a 1:1.000 dilution of the disinfectant composition of Example I for testing against animal viruses to determine viricidal activity. The disinfectant composition, at the 1:1.000 dilution, was tested against infectious Bovine Rhinotracheitis, Bovine Viral Diarrhea, Blue Tongue Virus and Porcine Parvo Virus. In all four instances, no viricidal activity was noted.

EXAMPLE V

The disinfectant composition was prepared according to the formula of Example I, above, and was packaged in 8 oz. polyethylene terephthalate bottles. A dairy with 800 cows was given somewhat more that 60 bottles of the composition to evaluate, and the product was used for about 2½ months. Using a 1:1.000 dilution, a foot bath was prepared, and cattle walked through the bath twice a day. The cattle exhibited no reluctance to passing through the bath, and there was no skin reaction or irritation within the 75-day period. The composition mixed well and, as stated by the dairy owner, was easier to handle than other similar products. Observations by the owner of the cattle indicated no "cure" for the clinical disease Hairy Foot Wart, but it was noted that spread of the disease was significantly reduced from cow to cow.

EXAMPLE VI

A final experiment was conducted to determine the effect, if any, of prolonged exposure to the disinfectant composition of this invention on the hooves of cattle, sheep and horses. Hooves from three cattle, three sheep and three horses were collected, cleaned and tagged as to species, sex, animal number and which hoof. The hooves were randomly placed into five study groups, with two hooves from each species being put into each group. The groups were identified by letters A-E and were classified by the solution used for soaking the hooves in that group, as follows:

GROUP	SOLUTION
A	10 ml disinfectant solution according to Example I with 10 liters water
B	20 ml disinfectant solution according to Example I with 10 liters water
C	3 lbs. copper sulfate dissolved in 3 gallons of water
D	Plain tap water
E	50 ml HOOFPRO+, as manufactured and sold by SSI Corporation with 10 liters water.

Before treatments with the solutions identified above, samples were removed from each hoof and tested for hardness using a PTC Instruments Durometer. After the initial samples were taken, the hooves were placed in plastic bags filled with the appropriate solution and sealed with as much air removed as possible. After 1 hour, the solutions were drained out and the hooves were placed on a rack to air dry. These soakings occurred 2

times each day with a minimum of 8 hours between soakings. The soakings continued for 14 days with samples collected on day 7 and day 14.

While all hooves showed increase in hardness from day 0 (no treatment) to day 14 of treatment, the hooves treated with the disinfectant composition of this invention (Groups A and B) in most instances fell within the ranges defined by the tests conducted with Groups C, D and E. In summary, the disinfectant composition of this invention exhibited no adverse effect on hoof hardness. Also evaluated was the composition of the hooves in regards to Nitrogen, Phoshporus, Potassium, zinc, iron, manganese, copper, calcium and magnesium on days 0, 7 and 14 during exposure. Results indicated no significant change in the test groups except for the copper sulfate which resulted in dramatic increases in copper content.

It is to be remembered that the above examples are provided for illustrative purposes only, and are not deemed to be limiting to the scope of the present invention. Results obtainable using pseudo-ionone rather than beta-ionone will be quite comparable to those stated above. Also, as previously indicated, the use of terpenes other than DL-limonene such as, for example, dipentene, citral, terpineol, and pinene exhibit similar results of increased efficacy over the use of ionone alone, and DL-limonene is the preferred other terpene because of its "generally regarded as safe" status. While it is contemplated that virtually any non-ionic surfactant having an HLB range of about 10 to about 18 is acceptable, polysorbates are preferred surfactants. It is also to be remembered that the use of isopropyl or ethyl alcohol is for the purpose of giving the final composition a clear appearance, and this alcohol may be omitted.

It will thus be seen that the objects set forth above, among those made apparent from the preceding description, are efficiently attained, and, since certain changes may be made in carrying out the above method and in the composition set forth without departing from the scope of the invention, it is intended that all matter contained in the above description shall be interpreted as illustrative and not in a limiting sense.

It is also to be understood that the following claims are intended to cover all of the generic and specific features of the invention herein described, and all statements of the scope of the invention which, as a matter of language, might be said to fall therebetween. Particularly, it is to be understood that in said claims, ingredients or compounds recited in the singular are intended to include compatible mixtures of such ingredients wherever the sense permits.

Now that the invention has been described,

What Is Claimed Is:

1. A disinfectant composition containing an ionone and another terpene, said composition comprising:

(a) from about 1% to about 70%, by volume, ionone selected from the group consisting of beta-ionone and pseudo-ionone:

(b) from about 1% to about 70%, by volume, other terpene selected from the group consisting of DL-limonene, dipentene, citral, terpineol, and pinene; and

(c) from about 1% to about 30%, by volume, surfactant selected from the group consisting of non-ionic surfactants having an HLB of about 10 to about 18.

2. A disinfectant composition as in claim 1 further comprising about 1-10%, by volume, alcohol selected from the group consisting of isoprophyl and ethyl alcohol..

3. A disinfectant composition as in claim 1 comprising about 45%, by volume, beta-ionone.

4. A disinfectant composition as in claim 1 comprising about 40%, by volume, DL-limonene.

5. A disinfectant composition as in claim 1 comprising about 10%, by volume, polysorbate 80.

6. A disinfectant composition containing an ionone and another terpene, said composition comprising:

(a) about 45%, by volume, ionone selected from the group consisting of beta-ionone and pseudo-ionone:

(b) about 40%, by volume, other terpene selected from the group consisting of DL-limonene, dipentene, citral, terpineol, and pinene;

(c) about 10%, by volume, surfactant selected from the group consisting of non-ionic surfactants having an HLB of about 10 to about 18; and

(d) about 5%, by volume, isopropyl or ethyl alcohol.

7. A disinfectant composition as in claim 6 wherein said ionone is beta-ionone.

8. A disinfectant composition as in claim 6 wherein said other terpene is DL-limonene.

9. A disinfectant composition as in claim 6 wherein said surfactant is polysorbate 80.

10. A disinfectant composition comprising:

(a) about 45%, by volume, beta-ionone;

(b) about 40%, by volume, DL-limonene;

(c) about 10%, by volume, polysorbate 80; and

(d) about 5%, by volume, isopropyl alcohol.

11. A method for preparing a disinfectant composition containing an ionone selected from the group consisting of beta-ionone and pseudo-ionone, another terpene selected from the group consisting of DL-limonene, dipentene, citral, terpineol, and pinene, a surfactant selected from the group consisting of non-ionic surfactants having an HLB range of about 10 to about 18, and isopropyl alcohol, said method comprising the steps of:

(a) adding about 45%, by volume, ionone to a mixing vessel maintained at about 20-25°C, with mixing;

(b) adding about 10%, by volume, surfactant to the ionone with additional mixing for about 60 minutes;

(c) adding about 5%, by volume, isopropyl alcohol to the mixture of step (b) at a rate of about 5-15 liters per minute with additional mixing for about 40 minutes;

(d) slowly adding about 40%, by volume, terpene with additional mixing while maintaining a substantially clear mixture; and

(e) blending the mixture of step (d) for about 1.5 hours to obtain the disinfectant composition.